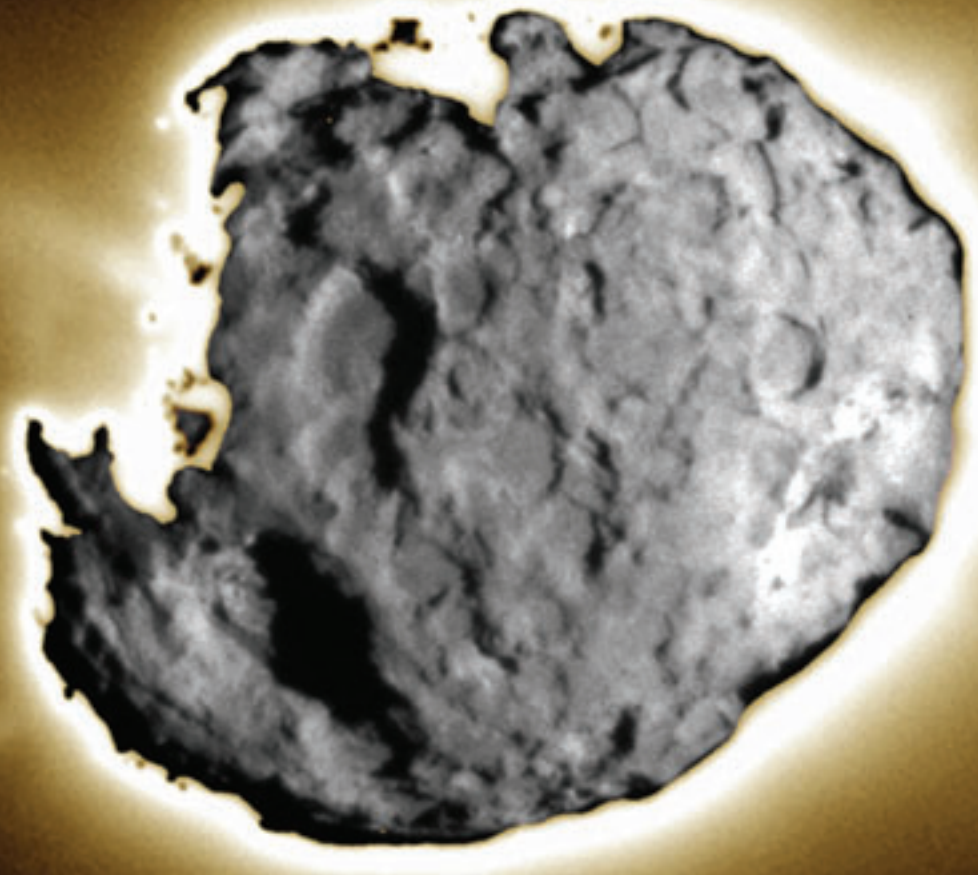
MICROSCOPY TODAY

JULY 2007 VOLUME 15 - NUMBER 4



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Hitachi S-5500

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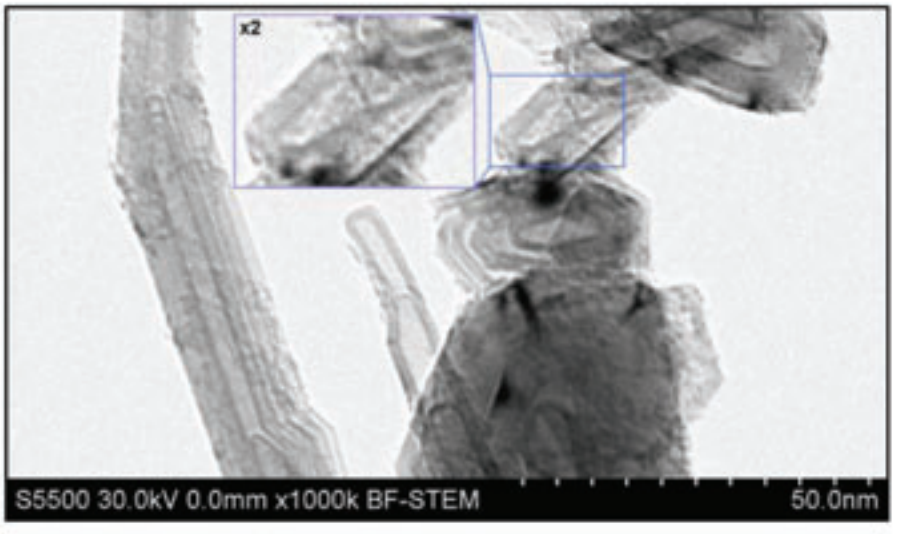
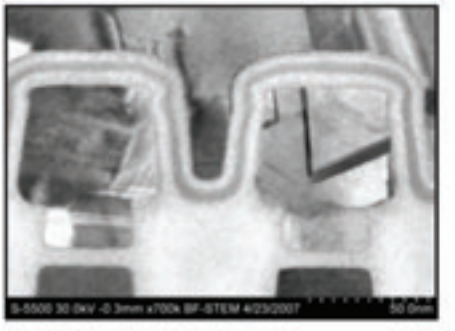
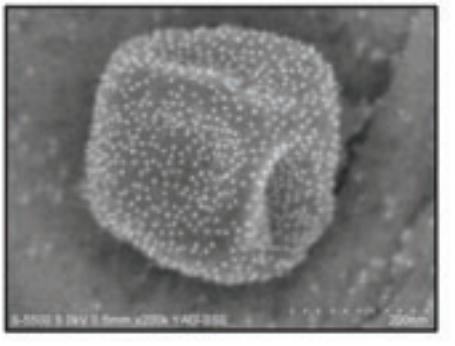


Fringes of MWCNT in STEM



NAND double-gate cross-section in STEM



Immuno Labeling

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Probing Individual Proteins in Unsupported Membranes

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Proteins in biologic membranes perform a large variety of essential functions. The fact that about one third of all genes code for membrane proteins, and that the majority of drugs target these proteins, attest to that fact. However, until now, proteins have been studied under artificial conditions, such as after being crystallized, frozen, or adsorbed to a substrate. Rui Pedro Gonçalves, Guillaume Agnus, Pierre Sens, Christine Houssin, Bernard Bartenlian, and Simon Scheuring have devised a novel setup with the atomic force microscope (AFM) to allow proteins to be probed while they are in unsupported membranes.[2] Their method is similar in principle to methods where a small area of a membrane is sampled, such as when a piece of membrane is examined by patch clamping. The difference is that instead of attaching a membrane to the end of a pipette, it is spread across a piece of perforated Si(001). This provided "viewing holes" in the range of 90 nm to 250 nm. With the AFM, the membranes could be imaged and manipulated while in an aqueous buffered medium at ambient pressure and temperature.

Gonçalves *et al*. chose the surface membrane of *Corynebacterium glutamicum* as their model for imaging studies because this membrane has been so well characterized in many different ways. Patches of membranes could be clearly seen to be spanning holes on the nano-patterned Si(001) support, yet proteins within patches were considerably smaller than the holes. Using a specific AFM mode, they visualized the subunit architecture of the proteins. Such structural details as a pore about 15Å in diameter, corresponding to a channel pore, could be clearly seen. In addition to imaging, force measurements could also be made on the unsupported membranes. Specifically, they could analyze forces as the AFM tip made contact with the membrane and when it pierced the membrane. Measuring forces between these two events provided interesting information on the lateral interaction force between proteins within the membrane that suggested fairly weak protein-protein interactions reinforcing the importance of cooperative interactions.

Using *Halobactrium salinarium* purple membranes, known to contain proton pumps, Gonçalves *et al*. isolated wells of about 10 attoliters beneath suspended membranes. These wells were primed with the *p*H-sensitive fluorescent probe pyranine. Using quantitative fluorescence microscopy, they were able to demonstrate a significant increase in *p*H under appropriate circumstances. This was clear evidence that the pumping of protons was being directly observed.

This novel technique is an extension of several methods for imaging and manipulating individual membrane proteins as reviewed by Gonçalves and Scheuring.[3] The key to their new method is that a membrane is suspended over a very small well. As techniques to manipulate fluids on a nano-scale are devised to mimic events occurring within cells, new methods can be developed to measure these events. Also, as AFMs become faster and more sensitive at acquiring images, these observations can be made at low physiologic levels and with better spatial and temporal resolution. We will be able to, in effect, see and measure what membrane proteins realistically do within cells with more precision than has ever been possible! ■

1. The author gratefully acknowledges Dr. Simon Scheuring for reviewing this article.
2. Gonçalves, R.P., G. Agnus, P. Sens, C. Houssin, B. Bartenlian, and S. Scheuring, Two-chamber AFM: probing membrane proteins separating two aqueous compartments, *Nature Methods* **3**:1007-1012, 2006.
3. Gonçalves, R.P., and S. Scheuring, Manipulating and imaging individual membrane proteins by AFM, *Surface and Interface Analysis* **38**:1413-1418, 2006.

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ABOUT THE COVER

This composite image was taken by the navigation camera during the close approach phase of Stardust's Jan 2, 2004 flyby of comet Wild 2. Several large depressed regions can be seen. Comet Wild 2 is about five kilometers (3.1 miles) in diameter. To create this image, a short exposure image showing tremendous surface detail was overlain on a long exposure image taken just 10 seconds later showing jets. Together, the images show an intensely active surface, jetting dust and gas streams into space and leaving a trail millions of kilometers long. See the article by Rietmeijer starting on page 6. Image credit: NASA/JPL-Caltech.

Interested readers may want to Google "wild 2 comet pictures" to find many more images of Wild 2, including stereo pairs.